Integrated Disinfection Byproducts Mixtures Research: Assessing Reproductive and Developmental Risks Posed by Complex Mixtures of Disinfection Byproducts

Glenn Rice, NCEA/ORD/U.S. EPA, Cincinnati, OH, Linda K. Teuschler, NCEA/ORD/U.S. EPA, Cincinnati, OH, Thomas E. Speth, NRMRL/ORD/U.S. EPA, Cincinnati, OH
Susan D. Richardson, NERL/ORD/USEPA, Athens, GA, Richard J. Miltner, NRMRL/ORD/U.S. EPA, Cincinnati, OH , Kathleen M. Schenck, NRMRL/ORD/U.S. EPA, Cincinnati, OH, Chris Gennings, Virginia Commonwealth University, Richmond, VA
E. Sidney Hunter III, NHEERL/ORD/U.S. EPA, Research Triangle Park, NC, Michael G. Narotsky, NHEERL/ORD/U.S. EPA, Research Triangle Park, NC, Jane Ellen Simmons, NHEERL/ORD/U.S. EPA, Research Triangle Park, NC

Chemical disinfection of drinking water causes the formation of complex chemical mixtures of disinfection by-products (DBPs). The composition of DBP mixtures is highly variable and includes compounds that have not been chemically identified. Chemical disinfection of drinking water results in nearly ubiquitous exposures to DBPs in recipients of treated water. In some epidemiologic studies, exposures to DBPs have been associated with increased risks of reproductive and developmental effects such as spontaneous abortions and low birth weights. Toxicologic studies of individual DBPs indicate that some DBPs may be reproductive and developmental toxicants. However, the overall evidence inconclusive. Although 11 DBPs are regulated in U.S. drinking waters, concerns persist about the toxicity of DBP mixtures and the possible need for additional regulations to reduce concentrations of additional DBPs. Four laboratories in the U.S. EPA's Office of Research and Development are jointly undertaking a study to evaluate the reproductive toxicity associated with concentrated DBP mixtures. The U.S. EPA is undertaking a study to evaluate the reproductive toxicity associated with concentrated DBP mixtures. This poster presents a toxicologically-based risk assessment strategy for identifying the individual components or fractions of a complex mixture that are associated with its toxicity. To illustrate the strategy, information is used on the toxicity of two concentrated whole mixtures of DBPs generated during the planning phase of the EPA's study. Analysis of these data suggests that alterations in experimental design may be needed if effects are to be observed. These may include an increase in DBP concentrations, changing the experimental strain of rat used, changing the bioassay utilized to evaluate toxicity of the mixture, and appropriately powering such a study. Finally, the importance of developing statistical and toxicological methods for evaluating the similarity of different mixtures based on component composition and component toxicity is identified. Collaborative efforts outside of U.S. EPA are being sought for key aspects of this study to enhance the understanding of the issues and to provide effective analysis.

Purpose

- Present Mixtures Risk Assessment Approach for EPA's concentrated DBP mixtures project
- Overview EPA's Concentrated DBP Mixture Project
- Present Risk Assessment Approach

Disinfection By-Product Exposure & Epidemiology

- DBP formation depends on many factors including:
  - —source water characteristics (e.g., Br-/Cl2, pH, type of NOM)
  - —disinfectant
  - —time in distribution system
- Most of U.S. population is exposed to DBPs
  - —Complex Mixture- highly variable component concentrations
    - Known, routinely measured DBPs
    - Unknown DBPs (e.g., halogenated compounds)
  - —Concurrent exposures to multiple chemicals over time
  - —Multiple routes of exposure
- Epi data: chlorinated tap water exposures may be risk factors for:
  - —spontaneous abortions, low birth weight & small for gestational age
- Roughly 25 of ~500 identified DBPs subjected to toxicologic study
- US regulates 2 Classes of DBPs (4 THMs and 5 HAAs)

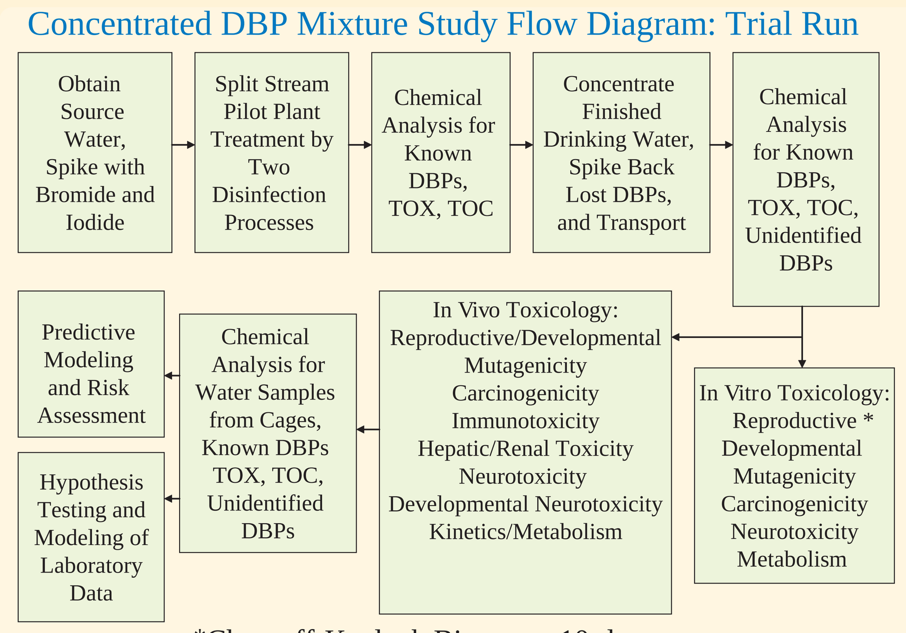
Concentrated DBP Mixtures Research Project Goals

- Generate health effects and chemical identity information for same chlorinated drinking water.
- Establish whether rodent whole-mixture DBP bioassays corroborate positive epidemiological studies
- Estimate fraction of toxicity attributable to different mixture components including unidentified DBPs
- Plan and execute full concentrated DBP Mixtures Study see Simmons et. al, 2002.

Questions Prompting Trial Run

- Feasibility of concentrating large quantities of regulatory compliant water in a biological matrix?
- Use of "spike back" procedures for volatile DBPs?
- Can chemical integrity of samples be maintained during experimental timeframe?
  - — transport, storage and time on animal cages
- Will test animals drink the concentrated water?
  - — Note: Trial Run - Not designed as thorough toxicological evaluation
- Can we compare risks associated with ozonation and chlorination?

Concentrated DBP Mixture Study Flow Diagram: Trial Run



*Chernoff-Kavlock Bioassay -10-day exposure

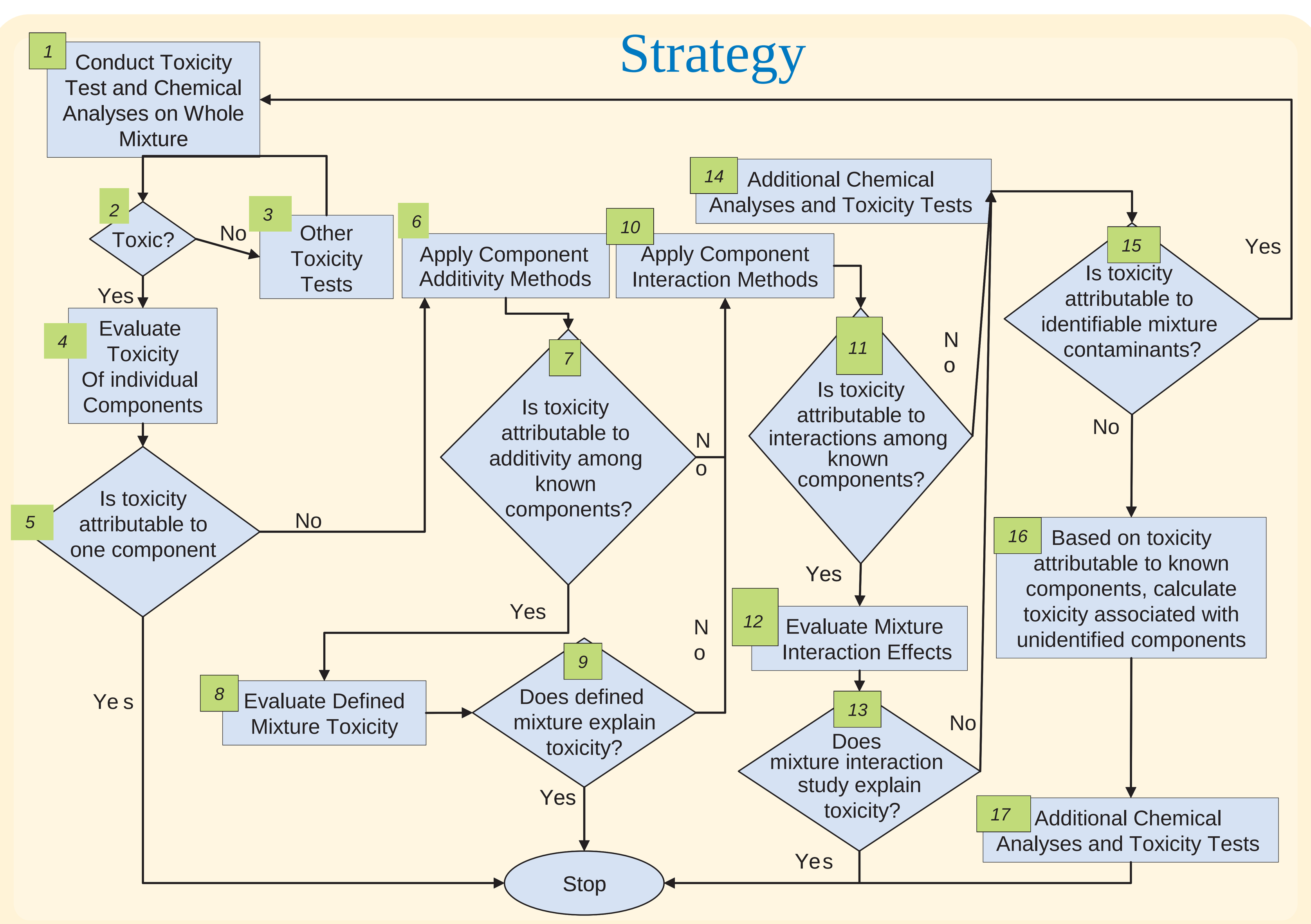
Selected Toxicology Results from Trial Run

- Rats drank water concentrates!
- Positive dose-response for in vitro mutagenicity assays
  - —regulated THMs only account for part of observed mutagenicity
  - —volatile DBPs mutagenic
- No detectable effects on maternal body weight in groups that drank the water concentrates
- With the exception of a statistically significantly effect on gestation length in ozonated group, reproductive/developmental effects were not detected in the Chernoff/Kavlock assay in 20 Sprague-Dawley dams and their PD-6 pups at DBP concentrations of ~130x

Moving Forward: How will we handle the data?

- Needed to evaluate the pilot data
- Needed an approach to identify which components or fractions are toxic if full study is positive
  - —Expected toxicity?
- Lack of recent approaches that evaluate which fractions of while mixtures are toxic in human health risk assessment.
- Developed a 4 Phase Risk Assessment Approach
  - —"top down" mixtures toxicity-based strategy for analyzing toxicity of complex mixture
  - —Confirm toxicity with defined mixtures, where possible
- Apply strategy to trial run data looking forward to full study

Strategy



Example: Apply Component Additivity Methods to Illustrate Strategy (see #6 in diagram)

- Is toxicity attributable to additivity among known components?
- If, so test with defined mixture

Dose Addition Theory

Fundamental Assumption:

- The components of a mixture must exhibit a common mode of toxic action.
- A theoretical consequence of the common mode of action assumption is that the dose-response functions of the components exhibit similar shapes between the response maxima and the threshold.
- In reality, toxicological assays of chemicals having a common mode of action may not exhibit similarly-shaped dose-response functions. e.g, kinetic differences, random error
- For two chemicals mixture response = sum(doses scaled for relative potency), evaluated using the D-R curve of the index chemical 1

$$R_m = f_1\left(D_1 + t * D_2\right)$$

Where: Rm = mixtures response
Di = exposure dose of chemical i
t = potency of chemical 2 relative to chemical 1
f1 = dose response function for index chemical 1

Illustrative RPF Application

- Index Chemical = Bromodichloromethane
- Toxicity- full-litter resorption (Narotsky et al., 1997) basis of dose response curve (f_1)
  - —different strain used in trial run
- RPF basis: Ratio of Repro or Developmental NOAELs (US EPA, 2000a)
  - —NOAELs for different effects
  - —Probability of "developmental effect", assuming that all DBPs in the mixture can be expressed as equivalent units of BDCM.
  - —Assume RPF of unidentified fractions = 1
  - —Assume no threshold

—P(Effect) = 1/(1+EXPONENTIAL(11.7-2.45 X ln(dose)))

RPF-Based Rodent Risk Estimates Based on DBP Concentration Levels in Concentrated Mixtures

	Post-Chlorination	
	mg/kg/day	P(Effect)
ICED	2.39	7×10^{-5}
ICED plus Unidentified TOX	4.04	3×10^{-4}
Unidentified Tox	1.65	3×10^{-5}
	Pre-Ozonation /Post -Chlorination	
	mg/kg/day	P(Effect)
ICED	1.72	3×10^{-5}
ICED plus Unidentified TOX	2.69	1×10^{-4}
Unidentified Tox	0.97	8×10^{-6}

ICED= index chemical equivalent dose
TOX= Total organic halogenated fraction

Concentrated DBP Mixtures Full Study: Next Steps

- Increased DBP concentrations in full study to increase power to observe an effect
- Experimental Strain with increased sensitivity to chemically-mediated pregnancy loss
- Multigenerational Repro/Developmental study
- Need for a positive DBP mixture control
- Need concentrated raw water control
- Use this approach to identify toxic DBP fraction(s)
- Additional Waters and Treatment Types in future

References

Gennings, C., P. Schwartz, W.H. Carter, Jr. and J.E. Simmons. 1997. Detection of departures from additivity in mixtures of many chemicals with a threshold model. J. Agric. Biol. Environ. Stat. 2: 198-211.

Narotsky, M.G., R.A. Pegram and R.J. Kavlock. 1997. Effect of dosing vehicle on the developmental toxicity of bromodichloromethane and carbon tetrachloride in rats. Fund. Appl. Toxicol. 40(1):30-36.

NRC (National Research Council). 1988. Complex Mixtures Methods for In Vivo Toxicity Testing. Committee on Methods for In Vivo Toxicity Testing of Complex Mixtures. Board on Environmental Studies and Toxicology, Commission on Life Sciences. National Academy Press, Washington, DC.

Simmons, J.E., S.D. Richardson, T.F. Speth et al. 2002. Development of a research strategy for integrated technology-based toxicological and chemical evaluation of complex mixtures of drinking water disinfection byproducts. Environ. Health Perspect. 110(6):1013-1024.

U.S. EPA. 2000a. Review of Animal Studies for Reproductive and Developmental Toxicity Assessment of Drinking Water Contaminants: Disinfection By-Products (DBPs). Prepared for the Office of Water by R.W. Tyl under contract with RTI, project # 07639.

U.S. EPA. 2000b. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. Office of Research and Development, Washington, DC. EPA/630/R-00/002. Available in pdf format at www.epa.gov/NCEA/raf/chem_mix.htm.